

WE CLAIM:

1. A process for amplifying at least one target nucleic acid sequence comprising:

5 (a) synthesizing a nucleic acid by hybridizing a primer complex to the sequence and extending the primer to form a first strand complementary to the sequence and a second strand complementary to the first strand, wherein the complex comprises a primer complementary to the sequence and a promoter region in an anti-sense orientation with respect to the sequence; and

10 (b) transcribing copies of anti-sense RNA off of the second strand.

15 2. A process according to Claim 1, wherein the primer complex comprises a promoter region from a T bacteriophage.

20 3. A process according to Claim 1, wherein the bacteriophage is T7 or T3. *a*

4. A process according to Claim 1, wherein the target nucleic acid sequence is mRNA.

25 5. A process according to Claim 1, wherein the RNA sequence has a poly(A) tail.

6. A process according to Claim 5, wherein the primer complex comprises a poly(T) primer sequence.

30 7. A process for detecting expression of a gene in a preselected cell population comprising:

35 (a) synthesizing double-stranded complementary deoxyribonucleic acid (cDNA) by treating mRNAs from the cell population with a primer complex comprising an oligonucleotide complementary to one or more of the RNA sequences, the primer linked to a promoter region in an orientation capable of directing transcription of anti-sense RNA;

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(b) transcribing the cDNA into anti-sense RNA by introducing an RNA polymerase capable of operably binding to the promoter region; and

(c) determining the presence or absence of transcribed anti-sense RNA complementary to mRNA corresponding to the gene.

8. A process according to Claim 7, wherein the cell population is from a human tissue.

9. A process according to Claim 7, wherein the tissue library is brain nuclei.

10. A process according to Claim 7, wherein the cell population comprises about 100 cells.

11. A process for producing a subtractive hybridization probe comprising:

(a) synthesizing a first double-stranded cDNA collection by treating a first mRNA population with a primer complex, wherein the primer is complementary to the RNA sequence and is operably linked to a first promoter region for transcription of the cDNA strand complementary to the primer;

(b) transcribing the first cDNA into anti-sense RNA by introducing a first RNA polymerase capable of binding to the first promoter region;

(c) hybridizing the anti-sense RNA to a second mRNA population, whereby an unhybridized subpopulation of the second RNA population is found;

(d) generating a second double-stranded cDNA collection from the unhybridized subpopulation using a second primer complex comprising a second promoter region in an orientation for transcribing anti-sense RNA complementary to the unhybridized subpopulation; and

(e) transcribing the second cDNA into a ribonucleotide probe by introducing a second RNA polymerase capable of binding to the second promoter region.

12. A method for making a cDNA library from a

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collection of mRNA molecules comprising:

(a) hybridizing one or more primer complexes to a plurality of the mRNA's, wherein each complex comprises an oligonucleotide primer linked to a promoter sequence capable of directing transcription of a DNA sequence complementary to the primer;

(b) producing a collection of double-stranded cDNAs by extending the primers of a plurality of any hybridization duplexes formed between the mRNA's and the complexes wherein each cDNA comprises a first strand complementary to one mRNA molecule and a second strand operably linked to the promoter sequence;

(c) transcribing multiple copies of anti-sense RNA off of the second strand; and

(d) preparing a cDNA library from the anti-sense RNA copies.

13. A method utilizing a promoter for amplifying a nucleic acid sequence comprising:

(a) binding to the nucleic acid sequence a primer operably linked to a promoter sequence capable of initiating transcription of RNA complementary to the nucleic acid sequence;

(b) synthesizing complementary DNA by elongation from the primer;

(c) extending the complementary DNA to generate a functional promoter; and

(d) adding RNA polymerase to initiate RNA synthesis from the promoter, whereby multiple copies of the nucleic acid sequence are produced.

14. A method according to Claim 13, wherein the promoter and polymerase are a cognate pair.

15. A method according to Claim 13, wherein the promoter is a naturally occurring prokaryotic promoter.

16. A method according to Claim 13, wherein the

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promoter is a bacteriophage promoter.

17. A method according to Claim 13, wherein the promoter is a T3, T5, or SP6 promoter.

5 18. A method according to Claim 13, wherein complementary DNA is synthesized by reverse transcriptase.

10 19. A method according to Claim 13, wherein the nucleic acid is RNA.

20. A method according to Claim 19, wherein the RNA sequence has a poly(A) tail.

15 21. A method according to Claim 19, wherein the primer comprises a poly(T) sequence.

22. A method for subtractive hybridization comprising the steps of:

20 (a) binding a primer to sense RNA molecules in a population, wherein the primer is operably linked to a promoter sequence in an anti-sense orientation;

(b) synthesizing complementary DNA by elongation from said primer;

25 (c) extending the complementary DNA to generate a functional promoter;

(d) initiating RNA synthesis from the promoter by adding RNA polymerase, whereby anti-sense RNA is produced;

30 (e) introducing the anti-sense RNA in molar excess to a second population of sense RNA molecules, whereby substantially complementary RNA sequences from the two populations hybridize; and

(f) isolating remaining single-stranded sense RNA.

35 23. A method according to Claim 22, wherein the amplified RNA is labeled during synthesis.

24. A method for detecting the expression of a gene

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in one or more cells, said method comprising the steps of:

(a) binding a primer to RNA molecules in a population, wherein the primer is operably linked to a promoter sequence in an anti-sense orientation;

(b) synthesizing complementary DNA by elongation from said primer;

(c) extending the complementary DNA to generate a functional promoter;

(d) initiating RNA synthesis from the promoter by adding RNA polymerase, thereby producing amplified RNA;

(e) analyzing the amplified RNA to determine expression of the gene.

25. The method according to Claim 24, wherein the amplified RNA is analyzed by hybridizing to a DNA sequence substantially corresponding to the gene to detect RNA copies of the gene.

26. A method according to Claim 25, wherein the DNA sequence is present on a Southern blot.

27. A method according to Claim 24, wherein the RNA is radioactively labeled during synthesis.

28. A method according to Claim 27, wherein the cell is obtained from human tissue.

29. A method according to Claim 24, wherein the cells comprise about 100 cells.

30. A method for amplifying mRNA in single brain cells comprising:

(a) introducing into the brain cells a reaction mixture comprising primer operably bound to promoter sequence in anti-sense orientation, reverse transcriptase, dATP, dCTP, dGTP, and dTTP thereby generating first strand cDNA;

(b) harvesting brain cell soma;

(c) precipitating nucleic acids from the brain cell

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soma;

(d) synthesizing second-strand cDNA by the addition of DNA polymerase;

(e) removing hairpin-loop structures in the cDNA by treating with S1 nuclease;

5 (f) synthesizing a functional promoter by adding T4 DNA polymerase, dATP, dCTP, dGTP and dTTP;

(g) initiating transcription by adding T7 RNA polymerase, rATP, rCTP, rGTP and UTP; whereby anti-sense RNA is produced.

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31. A component kit for the production of amplified RNA comprising: a container comprising a primer complex component and instructions for use of said primer complex.

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32. A component kit according to Claim 31, further comprising

(a) a container comprising a reverse transcriptase;

(b) a container comprising an RNA polymerase;

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(c) a container comprising dATP, dCTP, dGTP, and dTTP nucleotides; or

(d) a container comprising rATP, rCTP, rGTP, and UTP nucleotides.

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33. A component kit according to Claim 31, wherein the primer complex comprises a mixture of nucleic acid sequences.

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34. A component kit according to Claim 31, wherein the primer complex comprises a poly(T) primer capable of hybridizing to a collection of mRNA molecules.

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35. A composition comprising a collection of primer complexes, wherein a primer in one primer complex is poly(T)_n, wherein n is from about 5 to 50, or a population of degenerate primers.

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